

We claim:

1. A simplified method for simultaneous sequence-specific identification of multiple mRNA molecules in a RNA population comprising the steps of:
 - preparing a population of capturable double-stranded cDNA molecules from a population of mRNA molecules having a 3' poly (A) terminus by using a mixture of anchor primers, each anchor primer having a 5' terminus and a 3' terminus and including: (i) phasing residues located at the 3' terminus of each of the anchor primers selected from the group consisting of -V, -V-N, and -V-N-N, wherein V is a deoxyribonucleotide selected from the group consisting of A, C, and G; and N is a deoxyribonucleotide selected from the group consisting of A, C, G, and T, the mixture including anchor primers containing all possibilities for V and N where the anchor primer phasing residues in the mixture are defined by one of -V, -V-N, or -V-N-N; (ii) a tract of 8 to 40 T residues located towards the 5'-terminus relative to the phasing residues; (iii) a first stuffer segment consisting of 4 to 40 nucleotides; (iv) a segment complementary to a 3' PCR primer consisting of about 12 to about 20 nucleotide residues located towards the 5'-terminus relative to the tract of T residues; (v) a second stuffer segment consisting of 4 to 40 nucleotides and (vi) a capturable moiety affixed to the anchor primer;
 - digesting the population of capturable double-stranded cDNA molecules with a restriction endonuclease that recognizes at least a four nucleotide sequence not found within the sequence of the anchor primer, thereby producing a population of capturable double stranded cDNA fragments, each capturable double stranded cDNA fragment having an anchor end that corresponds to the poly(A) segment of the original mRNA molecule and including at least a portion of a sequence corresponding to that of the anchor primer, and a free end opposite to the anchor end;
 - capturing the capturable moiety, thereby affixing the capturable double-stranded cDNA fragments to a substrate to form affixed double stranded cDNA fragments;
 - ligating a double stranded adapter polynucleotide to the free end of each affixed double stranded cDNA fragment to form a population of adapted cDNA molecules, the double stranded adapter polynucleotide including a segment corresponding to the sequence of a bacteriophage RNA polymerase promoter and a segment complementary to a 5' PCR primer;

generating a first set of sequence-specific PCR products by dividing the population of adapted cDNA molecules into a first series of subpools as templates for a first polymerase chain reaction with a 3' PCR-primer about 15 to 30 nucleotides in length that is complementary to at least a portion of the anchor primer sequence and a first 5' PCR-primer about 15 to about 30 nucleotides in length and that is complementary to a portion of the adapter polynucleotide, with the complementarity extending one nucleotide beyond the portion of the adapter polynucleotide into the specific sequence corresponding to the free end of the capturable cDNA and including a 3'-terminus consisting of $-N_X$, wherein X is an integer from 1 to 5, and N is selected from group consisting of the four deoxyribonucleotides A, C, G, and T, and wherein a different one of the first 5' PCR primers is used in each of 4^X different subpools;

generating a detectable second set of sequence-specific PCR products by further dividing the first set of sequence-specific PCR products in each of the first series of subpools into a second series of subpools and using the first set of sequence-specific PCR products as templates for a second polymerase chain reaction with a 3' PCR primer of 15 to 30 nucleotides in length comprising a detectable moiety and a second 5' PCR primer defined as having a 3'-terminus consisting of $-N_X-N_{X+Y}$, wherein N_X is the same as the N_X used in the first polymerase chain reaction for that subpool, wherein Y is an integer from 1 to 5, $(X+Y)$ is an integer from 2 to 6, N is selected from group consisting of the four deoxyribonucleotides A, C, G, and T, wherein the second 5' PCR primer is about 15 to about 30 nucleotides in length and wherein the second 5' PCR-primer is complementary to a portion of the adapter polynucleotide with the complementarity extending $X+Y$ nucleotides beyond the portion of the adapter polynucleotide into the specific sequence corresponding to the free end of the capturable cDNA, wherein a different one of the second 5' PCR primers is used in the different 4^{X+Y} subpools of the second series of subpools;

resolving the second set of sequence-specific PCR products to generate a simultaneous display of sequence-specific PCR products representing the 3'-ends of mRNA molecules present in the mRNA population; and

characterizing each sequence-specific PCR product by a partial sequence and a length, thereby providing simultaneous sequence-specific identification of multiple mRNA molecules in a RNA population without making a cDNA library.

10. The method of claim 9 wherein the bacteriophage RNA polymerase promoter is a T3 promoter.

11. The method of claim 1 wherein one strand of the double stranded adapter polynucleotide comprises the sequence 5'-A-T-G-A-A-T-T-C-G-G-T-A-C-C-A-A-T-T-A-A-C-
5 C-C-T-C-A-C-T-A-A-A-G-G-G-A-C-A-G-C-T-T-A-T-C-A-T-C-G-C-T-C-G-A-G-C-T-C-G-A-C-G-G-T-A-T-3' (SEQ ID NO:7).

12. The method of claim 1 wherein one strand of the double stranded adapter polynucleotide comprises the sequence 5'-C-G-A-T-A-C-C-G-T-C-G-A-G-C-T-C-G-A-G-C-G-
10 A-T-G-A-T-A-A-G-C-T-G-T-C-C-C-T-T-A-G-T-G-A-G-G-G-T-T-A-A-T-T-G-G-T-A-C-C-G-A-A-T-T-C-A-T-3' (SEQ ID NO:8).

13. The method of claim 1 wherein one strand of the double stranded adapter polynucleotide comprises the sequence 5'-Phospho-C-G-A-T-A-C-C-G-T-C-G-A-C-C-T-C-G-
A-G-G-T-C-C-C-T-T-T-A-G-T-G-A-G-G-G-T-T-A-A-T-T-G-G-T-A-C-C-G-A-A-T-T-3' (SEQ ID NO:9).

14. The method of claim 1 wherein one strand of the double stranded adapter polynucleotide comprises the sequence 5'-A-A-T-T-C-G-G-T-A-C-C-A-A-T-T-A-A-C-C-C-T-
15 C-A-C-T-A-A-A-G-G-G-A-C-C-T-C-G-A-G-G-T-C-G-A-C-G-G-T-A-T-3' (SEQ ID NO:10).

15. The method of claim 1 wherein one strand of the double stranded adapter polynucleotide comprises the sequence 5'-Phospho-G-A-T-C-C-T-C-A-C-C-A-C-A-G-A-G-C-T-
20 T-C-G-A-G-G-T-C-C-C-T-T-T-A-G-T-G-A-G-G-G-T-T-A-A-T-T-G-G-T-A-C-C-G-A-A-T-T-3' (SEQ ID NO:11).

16. The method of claim 1 wherein one strand of the double stranded adapter polynucleotide comprises the sequence 5'-A-A-T-T-C-G-G-T-A-C-C-A-A-T-T-A-A-C-C-C-T-
25 C-A-C-T-A-A-A-G-G-G-A-C-C-T-C-G-A-A-G-C-T-C-T-G-T-G-G-T-G-A-G-3' (SEQ ID NO:12).

17. The method of claim 1 wherein one strand of the double stranded adapter polynucleotide comprises the sequence 5'-Phospho-C-T-C-A-C-C-A-C-A-G-A-G-C-T-T-C-G-A-
G-G-T-C-C-C-T-T-T-A-G-T-G-A-G-G-G-T-T-A-A-T-T-G-G-T-A-C-C-G-A-A-T-T-3' (SEQ ID NO:13).

18. The method of claim 1 wherein one strand of the double stranded adapter

polynucleotide comprises the sequence 5'-A-A-T-T-C-G-G-T-A-C-C-A-A-T-T-A-A-C-C-C-T-C-A-C-T-A-A-A-G-G-G-A-C-C-T-C-G-A-A-G-C-T-C-T-G-T-G-G-T-G-A-G-C-A-T-G-3' (SEQ ID NO:14).

19. The method of claim 1 wherein the 3' PCR primer comprises a sequence chosen from the group consisting of 5'-G-A-G-C-T-C-C-A-C-C-G-C-G-G-T-3' (SEQ ID NO:5)) and 5'-G-A-G-C-T-C-G-T-T-T-T-C-C-C-A-G-3' (SEQ ID NO:6).

20. The method of claim 4 wherein the RT primer comprises a sequence chosen from the group consisting of 5'- C-A-G-T-C-T-G-A-G-C-T-C-C-A-C-C-G-C-G-G-T-3' (SEQ ID NO:15) and 5'-G-A-G-C-T-C-C-A-C-C-G-C-G-G-T-3' (SEQ ID NO:5).

21. The method of claim 1 wherein the first 5' PCR primer comprises a sequence chosen from the group consisting of 5'-C-T-C-G-A-G-C-T-C-G-A-C-G-G-T-A-T-C-G-G-N-3' (SEQ ID NO:16), 5'-C-C-T-C-G-A-G-G-T-C-G-A-C-G-G-T-A-T-C-G-G-N-3' (SEQ ID NO:17), 5' A-G-C-T-C-T-G-T-G-G-T-G-A-G-G-A-T-C-N-3' (SEQ ID NO:19), 5'- A-G-C-T-C-T-G-T-G-G-T-G-A-G-C-A-T-G-N-3' (SEQ ID NO:21) and 5'- C-C-T-C-G-A-G-G-T-C-G-A-C-G-G-T-A-T-C-G-A-N -3' (SEQ ID NO:23).

22. The method of claim 1 wherein the second 5' PCR primer comprises a sequence chosen from the group consisting of 5'-C-G-A-C-G-G-T-A-T-C-G-G-N-N-N-N-3' (SEQ ID

NO:18), 5'-C-T-C-T-G-T-G-G-T-G-A-G-G-A-T-C-N-N-N-N-3' (SEQ ID NO:20), 5'-C-T-C-T-G-T-G-G-T-G-A-G-C-A-T-G-N-N-N-N-3' (SEQ ID NO:22), and 5'-T-C-G-A-G-G-T-C-G-A-C-G-G-T-A-T-C-G-A-N-N-N-N-3' (SEQ ID NO:24).

23. The method of claim 1 wherein the three nucleotides at the 3' end of the first 5' PCR primer are joined by phosphodiesterase-resistant linkages.

24. The method of claim 1 wherein the restriction endonuclease recognizing at least a four nucleotide sequence not found within the sequence of the anchor primer is selected from the group consisting of AciI, AluI, BfaI, BstUI, Csp6I, DpnI, DpnII, HaeIII, HhaI, HinPII, HpaII, MaeII, MboI, MnlI, MseI, MspI, NlaIII, RsaI, Sau3AI, TaiI, TaqI, and Tsp509I.

25. The method of claim 1 wherein the restriction endonuclease recognizing a four-nucleotide sequence is Msp I

26. The method of claim 1 wherein the restriction endonuclease recognizing at least a four nucleotide sequence not found within the sequence of the anchor primer is selected from the

group consisting of BglII, BmgI, BsaAI, BsaHI, BsaWI, BsbI, BsePI, BseSI, BsiI, BsiEI, BsiHKAI, BsiWI, BsmI, Bsp1286I, Bsp1407I, BspEI, BspGI, BspHI, BspLU11I, BspEI, BspGI, BspHI, BspLU11I, BspMII, BsrBI, BsrFI, BsrGI, BssHII, BssHIII, BssSI, BstBI, BstYI, BstZ17I, BtgI, BtrI, CfrI, Cfr10I, ClaI, DraI, DrdII, DsaI, EaeI, EagI, Ecl136II, Eco47III, EcoNI, EspI, Esp3I, FspI, GdiII, HaeI, HaeII, HgiAI, HgiEII, HgiJII, Hin4I, HincII, HindII, HindIII, KasI, KpnI, Ksp632I, LpnI, MfeI, MmeI, MscI, MslI, MspA1I, MstI, NaeI, NarI, NcoI, NdeI, NheI, Nli3877I, NotI, NruI, NspBII, OliI, PciI, PflMI, PmeI, Ppu10I, PpuMI, PspOMI, PsrI, PssI, PvuII, RleAI, RsrII, SapI, SauI, SbfI, SciI, SduI, SfcI, SfoI, SgfI, SgrAI, SmaI, SmlI, SnaI, SnaBI, SrfI, Sse232I, Sse8387I, Sse8647I, StyI, Tth111I, Tth111II, UbaKI, VspI, XbaI, XcmI, XhoI, XmaI.

27. The method of claim 1 wherein the restriction endonuclease recognizing at least a four nucleotide sequence not found within the sequence of the anchor prime is selected from the group consisting of AscI, BaeI, FseI, NotI, PacI, PmeI, PpuMI, RsrII, SapI, SexAI, SfiI, SgfI, SgrAI, SrfI, Sse8387I and SwaI.

28. The method of claim 4 wherein the restriction endonuclease that recognizes at least six bases is selected from the group consisting of AscI, BaeI, FseI, NotI, PacI, PmeI, PpuMI, RsrII, SapI, SexAI, SfiI, SgfI, SgrAI, SrfI, Sse8387I and SwaI.

29. The method of claim 4 wherein the restriction endonuclease that recognizes at least six bases is selected from the group consisting of EcoRI and XbaI.

30. The method of claim 1, wherein the capturable moiety of the anchor primer of the double stranded DNA molecule is affixed to a substrate comprising a coating selected from the group consisting of streptavidin, avidin, neutravidin, N-oxysuccinimide ester, dimethyladipimide-2-HCl, dimethylpimelimide-HCl, dimethylsuberimide-2HCl, dimethyl 3,3'-dithiobispropionimide-2HCl, disuccinimidyl glutarate, disuccinimidyl suberate, bis(sulfosuccinimidyl)suberate, dithiobis(succinimidyl propionate), dithiobis(sulfosuccinimidyl propionate), ethylene glycobis(succinimidylsuccinate), ethylene glycobis(sulfosuccinimidylsuccinate), disuccinimidyl tartarate, disulfosuccinimidyl tartarate, bis[2-succinimidylloxycarbonyloxy]ethyl sulfone, bis[2-(sulfosuccinimidylloxycarbonyloxy)ethyl] sulfone, and N-hydroxysuccinimidyl 2,3-dibromopropionate, succinimidyl 4-(N-maleimidomethyl) cyclohexane-1-carboxylate, sulfosuccinimidyl 4-(N-maleimidomethyl)

cyclohexane-1-carboxylate, m-maleimidobenzoyl-N-hydroxysuccinimide ester, m-maleimidobenzoyl-N-hydroxysulfosuccinimide ester, succinimidyl 4-(p-maleimidophenyl)-butyrate, sulfosuccinimidyl 4-(p-maleimidophenyl)-butyrate, bismaleimido-hexane, N-(γ -maleimidobutyryloxy)succinimide ester, N-(γ -maleimidobutyryloxy)sulfosuccinimide ester, N-succinimidyl(4-iodoacetyl)aminobenzoate, sulfosuccinimidyl(4-iodoacetyl)aminobenzoate, 1,4-Di-[3'-2'-pyridyldithio(propionamido)butane], 4-succinimidylloxycarbonyl- α -(2-pyridyldithio)toluene, sulfosuccinimidyl-6-[α -methyl- α -(2-pyridyldithio)-toluamido]hexane, N-succinimidyl-3-(2-pyridyldithio)-propionate, succinimidyl 6-[3-(2-pyridyldithio)propionamido]hexanoate, sulfosuccinimidyl 6-[3-(2-pyridyldithio)propionamido]hexanoate, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride, N,N'-dicyclohexylcarbodiimide, 1,5-difluoro-2,4-dinitrobenzene, N-5-azido-2-nitrobenzoyloxysuccinimide, N-hydroxysuccinimidyl-4-azidobenzoate, N-hydroxysulfosuccinimidyl-4-azidobenzoate, N-hydroxysuccinimidyl-4-azidosalicylic acid, N-hydroxysulfosuccinimidyl-4-azidosalicylic acid, sulfosuccinimidyl-(4-azidosalicylamido)-hexanoate, p-nitrophenyl-2-diazo-3,3,3-trifluoropropionate, 2-diazo-3,3,3-trifluoropropionylchloride, N-succinimidyl-(4-azidophenyl)1,3'-dithiopropionate, sulfosuccinimidyl-(4-azidophenyldithio)propionate, sulfosuccinimidyl 2-(7-azido-4-methylcoumarin-3-acetamide) ethyl-1,3'-dithiopropionate, sulfosuccinimidyl 7-azido-4-methylcoumarin-3-acetate, sulfosuccinimidyl 2(m-azido-o-nitrobenzamido)-ethyl-1,3'-dithiopropionate, N-succinimidyl-6-(4'-azido-2'-nitrophenylamino)hexanoate, sulfosuccinimidyl-6-(4'-azido-2'-nitrophenylamino)hexanoate, sulfosuccinimidyl 2-(p-azidosalicylamido)ethyl-1,3'-dithiopropionate, and sulfosuccinimidyl 4-(p-azidophenyl)butyrate and mixtures thereof.

31. The method of claim 30, wherein the capturable moiety of the anchor primer of the double stranded DNA molecule is affixed to a substrate comprising a coating of streptavidin.

32. The method of claim 30, wherein the capturable moiety of the anchor primer of the double stranded DNA molecule is affixed to a substrate comprising a coating of N-oxy-succinimide ester.

33. A method for simultaneous sequence-specific identification of multiple mRNA molecules in a RNA population comprising the steps of:

preparing a population of capturable double-stranded cDNA molecules from a population of mRNA molecules having a 3' poly (A) terminus by using a mixture of anchor primers, each anchor primer having a 5' terminus and a 3' terminus and including: (i) phasing residues located at the 3' terminus of each of the anchor primers consisting of -V-N-N, wherein V is a

5 deoxyribonucleotide selected from the group consisting of A, C, and G; and N is a deoxyribonucleotide selected from the group consisting of A, C, G, and T, the mixture including anchor primers containing all possibilities for V and N; (ii) a tract of 8 to 40 T residues located towards the 5'-terminus relative to the phasing residues; (iii) a first stuffer segment consisting of 4 to 40 nucleotides; (iv) a segment complementary to a 3' PCR primer consisting of about 12 to
10 about 20 nucleotide residues located towards the 5'-terminus relative to the tract of T residues; (v) a second stuffer segment consisting of 4 to 40 nucleotides; (vi) at least one segment comprising a sequence recognized by a restriction endonuclease that recognizes at least six bases, the segment being located towards the 5'-terminus of the anchor primer relative to the 3' PCR primer segment and (vii) a capturable moiety affixed to the anchor primer;

15 digesting the population of capturable double-stranded cDNA molecules with a restriction endonuclease that recognizes at least a four nucleotide sequence not found within the sequence of the anchor primer, thereby producing a population of capturable double stranded cDNA fragments, each capturable double stranded cDNA fragment having an anchor end that corresponds to the poly(A) segment of the original mRNA molecule and including at least a
20 portion of a sequence corresponding to that of the anchor primer, and a free end opposite to the anchor end;

capturing the capturable moiety, thereby affixing the capturable double-stranded cDNA fragments to a substrate to form affixed double stranded cDNA fragments;

ligating a double stranded adapter polynucleotide to the free end of each affixed double
25 stranded cDNA fragment to form a population of adapted cDNA molecules, the double stranded adapter polynucleotide including a segment corresponding to the sequence of a bacteriophage RNA polymerase promoter and a segment complementary to a 5' PCR primer;

generating a first set of sequence-specific PCR products by dividing the population of adapted cDNA molecules into a first series of subpools as templates for a first polymerase chain
30 reaction with a 3' PCR-primer about 15 to 30 nucleotides in length that is complementary to at

least a portion of the anchor primer sequence and a first 5' PCR-primer about 15 to about 30 nucleotides in length and that is complementary to a portion of the adapter polynucleotide, with the complementarity extending one nucleotide beyond the portion of the adapter polynucleotide into the specific sequence corresponding to the free end of the capturable cDNA and including a 3'-terminus consisting of $-N_X$, wherein X is an integer from 1 to 5, and N is selected from group consisting of the four deoxyribonucleotides A, C, G, and T, and wherein a different one of the first 5' PCR primers is used in each of 4^X different subpools;

generating a detectable second set of sequence-specific PCR products by further dividing the first set of sequence-specific PCR products in each of the first series of subpools into a second series of subpools and using the first set of sequence-specific PCR products as templates for a second polymerase chain reaction with a 3' PCR primer of 15 to 30 nucleotides in length comprising a detectable moiety and a second 5' PCR primer defined as having a 3'-terminus consisting of $-N_X-N_{X+Y}$, wherein N_X is the same as the N_X used in the first polymerase chain reaction for that subpool, wherein Y is an integer from 1 to 5, $(X+Y)$ is an integer from 2 to 6, N is selected from group consisting of the four deoxyribonucleotides A, C, G, and T, wherein the second 5' PCR primer is about 15 to about 30 nucleotides in length and wherein the second 5' PCR-primer is complementary to a portion of the adapter polynucleotide with the complementarity extending $X+Y$ nucleotides beyond the portion of the adapter polynucleotide into the specific sequence corresponding to the free end of the capturable cDNA, wherein a different one of the second 5' PCR primers is used in the different 4^{X+Y} subpools of the second series of subpools;

resolving the second set of sequence-specific PCR products to generate a simultaneous display of sequence-specific PCR products representing the 3'-ends of mRNA molecules present in the mRNA population; and

characterizing each sequence-specific PCR product by a partial sequence and a length, thereby providing simultaneous sequence-specific identification of multiple mRNA molecules in a RNA population without making a cDNA library.

34. The method of claim 33, wherein the step of preparing a population of double-stranded cDNA molecules comprises the steps of synthesizing a first cDNA strand and synthesizing a second cDNA strand.

35. The method of claim 33, further comprising the steps of transcribing to produce synthetic RNA molecules by incubating the capturable cDNA with a bacteriophage RNA polymerase capable of initiating transcription from the sequence corresponding to the sequence of a bacteriophage RNA polymerase promoter; and

36. The method of claim 33, wherein $X = 1$ and $Y = 3$.

10 consisting of primers having the sequence 5'-A-T-G-A-A-T-T-C-T-C-T-A-G-A-G-A-T-T-G-C-
T-A-C-C-T-C-A-G-T-C-T-G-A-G-C-T-C-C-A-C-C-G-C-G-G-T-A-G-T-A-C-T-C-A-C-T-G-C-
T-T-T-T-T-T-T-T-T-T-T-T-T-T-T-T-V-N-N-3' (SEQ ID NO: 1), 5'-A-T-G-A-A-T-T-C-T-
C-T-A-G-A-G-T-C-T-G-A-G-C-T-C-C-A-C-C-G-C-G-G-T-A-G-T-A-C-T-C-A-C-T-G-C-A-G-
T-T-T-T-T-T-T-T-T-T-T-T-T-T-T-T-V-N-N-3' (SEQ ID NO: 2) and 5'-G-A-A-T-T-C-A-A-
15 C-T-G-G-A-A-G-C-G-G-C-C-G-C-A-G-G-A-A-G-A-G-C-T-C-C-A-C-C-G-C-G-G-T-A-G-T-
A-C-T-C-A-C-T-G-C-A-G-T-V-N-N-3' (SEQ ID NO:
3), wherein the 5' terminal base (base 1) is a biotinylated adenylate residue, each V can represent
A, C or G, and each N can represent A, C, G, or T.

39 The method of claim 33 wherein one strand of the double stranded adapter polynucleotide comprises the sequence 5'-A-T-G-A-A-T-T-C-G-G-T-A-C-C-A-A-T-T-A-A-C-C-C-T-C-A-C-T-A-A-A-G-G-G-A-C-A-G-C-T-T-A-T-C-A-T-C-G-C-T-C-G-A-G-C-T-C-G-A-C-G-G-T-A-T-3' (SEQ ID NO:7).

41. The method of claim 33 wherein one strand of the double stranded adapter polynucleotide comprises the sequence 5'-Phospho-C-G-A-T-A-C-C-G-T-C-G-A-C-C-T-C-G-A-G-G-T-C-C-C-T-T-T-A-G-T-G-A-G-G-G-T-T-A-A-T-T-G-G-T-A-C-C-G-A-A-T-T-3' (SEQ ID NO:9).

5 42. The method of claim 33 wherein one strand of the double stranded adapter polynucleotide comprises the sequence 5'-A-A-T-T-C-G-G-T-A-C-C-A-A-T-T-A-A-C-C-C-T-C-A-C-T-A-A-A-G-G-G-A-C-C-T-C-G-A-G-G-T-C-G-A-C-G-G-T-A-T-3' (SEQ ID NO:10).

43. The method of claim 33 wherein one strand of the double stranded adapter polynucleotide comprises the sequence 5'-Phospho-G-A-T-C-C-T-C-A-C-C-A-C-A-G-A-G-C-T-10 T-C-G-A-G-G-T-C-C-C-T-T-T-A-G-T-G-A-G-G-G-T-T-A-A-T-T-G-G-T-A-C-C-G-A-A-T-T-3' (SEQ ID NO:11).

44. The method of claim 33 wherein one strand of the double stranded adapter polynucleotide comprises the sequence 5'-A-A-T-T-C-G-G-T-A-C-C-A-A-T-T-A-A-C-C-C-T-15 C-A-C-T-A-A-A-G-G-G-A-C-C-T-C-G-A-A-G-C-T-C-T-G-T-G-G-T-G-A-G-3' (SEQ ID NO:12).

45. The method of claim 33 wherein one strand of the double stranded adapter polynucleotide comprises the sequence 5'-Phospho-C-T-C-A-C-C-A-C-A-G-A-G-C-T-T-C-G-A-20 G-G-T-C-C-C-T-T-T-A-G-T-G-A-G-G-G-T-T-A-A-T-T-G-G-T-A-C-C-G-A-A-T-T-3' (SEQ ID NO:13).

46. The method of claim 33 wherein one strand of the double stranded adapter polynucleotide comprises the sequence 5'-A-A-T-T-C-G-G-T-A-C-C-A-A-T-T-A-A-C-C-C-T-25 C-A-C-T-A-A-A-G-G-G-A-C-C-T-C-G-A-A-G-C-T-C-T-G-T-G-G-T-G-A-G-C-A-T-G-3' (SEQ ID NO:14).

47. The method of claim 33 wherein the 3' PCR primer comprises a sequence chosen from the group consisting of 5'-G-A-G-C-T-C-C-A-C-C-G-C-G-G-T-3' (SEQ ID NO:5)) and 5'-G-A-G-C-T-C-G-T-T-T-T-C-C-C-A-G-3' (SEQ ID NO:6).

48. The method of claim 33 wherein the RT primer comprises a sequence chosen from the group consisting of 5'- C-A-G-T-C-T-G-A-G-C-T-C-C-A-C-C-G-C-G-G-T-3' (SEQ ID NO:15) and 5'-G-A-G-C-T-C-C-A-C-C-G-C-G-G-T-3' (SEQ ID NO:5).

49. The method of claim 33 wherein the first 5' PCR primer comprises a sequence

chosen from the group consisting of 5'-C-T-C-G-A-G-C-T-C-G-A-C-G-G-T-A-T-C-G-G-N-3' (SEQ ID NO:16), 5'-C-C-T-C-G-A-G-G-T-C-G-A-C-G-G-T-A-T-C-G-G-N-3' (SEQ ID NO:17), 5'-A-G-C-T-C-T-G-T-G-G-T-G-A-G-G-A-T-C-N-3' (SEQ ID NO:19), 5'-A-G-C-T-C-T-G-T-G-G-T-G-A-G-C-A-T-G-N-3' (SEQ ID NO:21) and 5'-C-C-T-C-G-A-G-G-T-C-G-A-C-G-G-T-A-T-C-G-A-N-3' (SEQ ID NO:23).

50. The method of claim 33 wherein the second 5' PCR primer comprises a sequence chosen from the group consisting of 5'-C-G-A-C-G-G-T-A-T-C-G-G-N-N-N-N-3' (SEQ ID NO:18), 5'-C-T-C-T-G-T-G-G-T-G-A-G-G-A-T-C-N-N-N-N-3' (SEQ ID NO:20), 5'-C-T-C-T-G-T-G-G-T-G-A-G-C-A-T-G-N-N-N-N-3' (SEQ ID NO:22), and 5'-T-C-G-A-G-G-T-C-G-A-C-G-G-T-A-T-C-G-A-N-N-N-N-3' (SEQ ID NO:24).

51. The method of claim 33 wherein the restriction endonuclease recognizing at least a four nucleotide sequence not found within the sequence of the anchor primer is selected from the group consisting of AciI, AluI, BfaI, BstUI, Csp6I, DpnI, DpnII, HaeIII, HhaI, HinPII, HpaII, MaeII, MboI, MnII, MseI, MspI, NlaIII, RsaI, Sau3AI, TaiI, TaqI, and Tsp509I.

52. The method of claim 33 wherein the restriction endonuclease recognizing a four-nucleotide sequence is Msp I

53. The method of claim 33 wherein the restriction endonuclease recognizing at least a four nucleotide sequence not found within the sequence of the anchor primer is selected from the group consisting of BglII, BmgI, BsaAI, BsaHI, BsaWI, BsbI, BsePI, BseSI, BsiI, BsiEI, BsiHKAI, BsiWI, BsmI, Bsp1286I, Bsp1407I, BspEI, BspGI, BspHI, BspLU11I, BspEI, BspGI, BspHI, BspLU11I, BspMII, BsrBI, BsrFI, BsrGI, BssHII, BssHII, BssSI, BstBI, BstYI, BstZ17I, BtgI, BtrI, CfrI, Cfr10I, ClaI, DraI, DrdII, DsaI, EaeI, EagI, Ecl136II, Eco47III, EcoNI, EspI, Esp3I, FspI, GdiII, HaeI, HaeII, HgiAI, HgiEII, HgiJII, Hin4I, HincII, HindII, HindIII, KasI, KpnI, Ksp632I, LpnI, MfeI, MmeI, MscI, MslI, MspA1I, MstI, NaeI, NarI, NcoI, NdeI, NheI, Nli3877I, NotI, NruI, NspBII, OliI, PciI, PflMI, PmeI, Ppu10I, PpuMI, PspOMI, PsrI, PssI, PvuII, RleAI, RsrII, SapI, SauI, SbfI, SciI, SduI, SfcI, SfoI, Sgfi, SgrAI, SmaI, SmlI, SnaI, SnaBI, SrfI, Sse232I, Sse8387I, Sse8647I, StyI, Tth111I, Tth111II, UbaKI, VspI, XbaI, XcmI, XhoI, XmaI.

54. The method of claim 33 wherein the restriction endonuclease recognizing at least a four nucleotide sequence not found within the sequence of the anchor prime is selected from the

group consisting of AscI, BaeI, FseI, NotI, PacI, PmeI, PpuMI, RsrII, SapI, SexAI, SfiI, Sgfl, SgrAI, Srfl, Sse8387I and SwaI.

55. The method of claim 33 wherein the restriction endonuclease that recognizes at least six bases is selected from the group consisting of AscI, BaeI, FseI, NotI, PacI, PmeI,

5 PpuMI, RsrII, SapI, SexAI, SfiI, Sgfl, SgrAI, Srfl, Sse8387I and SwaI.

56. The method of claim 33 wherein the restriction endonuclease that recognizes at least six bases is selected from the group consisting of EcoRI and XbaI.

57. The method of claim 33 wherein the capturable moiety of the anchor primer of the double stranded DNA molecule is biotin.

10 58. The method of claim 33 wherein the detectable moiety of the anchor primer of the second set of sequence-specific PCR products is a fluorescent moiety and is detected by monitoring laser-induced fluorescent emission.

15 59. The method of claim 33 wherein the restriction endonuclease that recognizes at least a four nucleotide sequence not found within the sequence of the anchor primer is MspI and the first 5' PCR primer comprises the sequence 5'-C-T-C-G-A-G-C-T-C-G-A-C-G-G-T-A-T-C-G-G-N-3' (SEQ ID NO:16).

20 60. The method of claim 33 wherein the first 5' PCR primer comprises the sequence 5'-C-C-T-C-G-A-G-G-T-C-G-A-C-G-G-T-A-T-C-G-G-N-3' (SEQ ID NO:17) and the second 5' PCR primer comprises the sequence 5'-C-G-A-C-G-G-T-A-T-C-G-G-N-N-N-N-3' (SEQ ID NO:18).

25 61. The method of claim 33 wherein the restriction endonuclease that recognizes at least a four nucleotide sequence not found within the sequence of the anchor primer is Sau3AI, the first 5' PCR primer comprises the sequence 5' A-G-C-T-C-T-G-T-G-G-T-G-A-G-G-A-T-C-N-3' (SEQ ID NO:19), and the second 5' PCR primer comprises the sequence 5'-C-T-C-T-G-T-G-G-T-G-A-G-G-A-T-C-N-N-N-N-3' (SEQ ID NO:20).

30 62. The method of claim 33 wherein the restriction endonuclease that recognizes at least a four nucleotide sequence not found within the sequence of the anchor primer is NlaIII, the first 5' PCR primer comprises the sequence 5'- A-G-C-T-C-T-G-T-G-G-T-G-A-G-C-A-T-G-N-3' (SEQ ID NO:21), and the second 5' PCR primer comprises the sequence 5'-C-T-C-T-G-T-G-G-T-G-A-G-C-A-T-G-N-N-N-N-3' (SEQ ID NO:22).

63. The method of claim 33 wherein the restriction endonuclease that recognizes at least a four nucleotide sequence not found within the sequence of the anchor primer is TagI, the first 5' PCR primer comprises the sequence 5'- C-C-T-C-G-A-G-G-T-C-G-A-C-G-G-T-A-T-C-G-A-N -3' (SEQ ID NO:23), the second 5' PCR primer comprises the sequence 5'- T-C-G-A-G-G-T-C-G-A-C-G-G-T-A-T-C-G-A-N-N-N-N-3' (SEQ ID NO:24).

64. A set of anchor primers having the sequence 5'-A-T-G-A-A-T-T-C-T-C-T-A-G-A-G-A-T-T-G-C-T-A-C-C-T-C-A-G-T-C-T-G-A-G-C-T-C-C-A-C-C-G-C-G-G-T-A-G-T-A-C-T-C-A-C-T-G-C-T-T-T-T-T-T-T-T-T-T-T-T-T-T-T-V-N-N-3' (SEQ ID NO: 1) wherein the 5' terminal base (base 1) is a biotinylated adenylate residue, V can represent A, C or G, and each N can represent A, C, G, or T.

65. A set of anchor primers having the sequence 5'-A-T-G-A-A-T-T-C-T-C-T-A-G-A-G-T-C-T-G-A-G-C-T-C-C-A-C-C-G-C-G-G-T-A-G-T-A-C-T-C-A-C-T-G-C-A-G-T-T-T-T-T-T-T-T-T-T-T-T-T-T-T-T-V-N-N-3' (SEQ ID NO: 2) wherein the 5' terminal base (base 1) is a biotinylated adenylate residue, V can represent A, C or G, and each N can represent A, C, G, or T.

66. A set of anchor primers having the sequence 5'-G-A-A-T-T-C-A-A-C-T-G-G-A-A-G-C-G-G-C-C-G-C-A-G-G-A-A-G-A-G-C-T-C-C-A-C-C-G-C-G-G-T-A-G-T-A-C-T-C-A-C-T-G-C-A-G-T-T-T-T-T-T-T-T-T-T-T-T-T-T-T-V-N-N-3' (SEQ ID NO: 3) wherein the 5' terminal base (base 1) is a biotinylated guanosine residue, V can represent A, C or G, and each N can represent A, C, G, or T.

67. A system for storing and displaying characteristics of polynucleotide fragments comprising, in combination:

a graphical user interface for visually displaying characteristics of polynucleotide fragments and

at least one database for storing characteristics of polynucleotide fragments stored on a computer readable medium.

68. The system of claim 67 wherein at least one database comprises data produced by the quantitation of the fragment length and relative abundance of sequence-specific PCR products.

69. The system of claim 68 wherein the sequence-specific PCR products are

differentially expressed sequence-specific PCR products.

70. The system of claim 67 wherein at least one database comprises data including sequence relationships, gene mapping and cellular distributions.

5 71. The system of claim 67 wherein at least one database comprises data including corresponding nucleotide sequences determined from a general database of nucleotide sequences, the corresponding nucleotide sequences being delimited by the 3'-most recognition site for the restriction endonuclease that recognizes at least a four nucleotide sequence not found within the sequence of the anchor primer and the beginning of the poly(A) tail.

10 72. The system of claim 71 wherein at least one database comprises data including an expected length of a sequence-specific PCR product.

15 73. The system of claim 72 wherein the expected length of a sequence-specific PCR product is determined from the sum of the lengths of the corresponding nucleotide sequence determined from the general database, the length of the 5' PCR sequence hybridizable to the adapter sequence, and the length of the remaining anchor primer sequence, including the length of the 3' PCR primer segment.

74. The system of claim 73 further comprising a set of executable instructions stored on a computer readable medium suitable for comparing characteristics of the sequence-specific PCR products to characteristics of the corresponding nucleotide sequence determined from the general database.